A Novel Expansion Process Enhances Tumor-Infiltrating Lymphocyte (TIL) Polyfunctionality, Cytotoxicity, and Expansion, While Preserving Cells in a Less Differentiated and More Stem-Like Phenotype

Andrew Yuhas, Joe Dean, Tim Erickson, Marcus Machin, Mohammed Alkhouli, Joe Yglesias, Rafael Cubas

Iovance Biotherapeutics, Inc., San Carlos, CA, USA

Corresponding Author: Rafael Cubas (Rafael.Cubas@Iovance.com)

Background

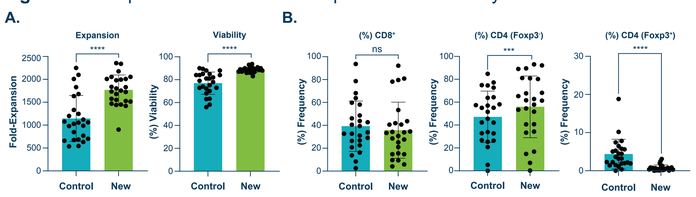
- Adoptive cell therapy using autologous tumor-infiltrating lymphocytes (TIL) has shown durable responses in patients with metastatic melanoma and various epithelial malignancies[1–3]
- Data suggest that a higher proportion of less differentiated and more stem-like TIL can be associated with persistence and response in patients with metastatic melanoma[4–5]
- Strategies to expand TIL in a process that preserves stem-like attributes while reducing cell differentiation may result in improved persistence, functionality, and response to TIL cell therapy
- Here, we describe a novel TIL expansion process that increases yield while preserving cells in a less-differentiated and more stem-like phenotype with enhanced functional output and cytotoxic capacity

Methods

- Tissue from various solid tumor histologies (eg, non-small cell lung cancer [NSCLC], renal cell carcinoma, breast cancer) (N=25) was fragmented and expanded using either a standard 22-day process (“control”) or a newly developed process (“new”) specifically designed to generate TIL with stem-like memory attributes and increased functional output
- Compared with control, the new process uses a different combination of cytokines during the pre-rapid expansion protocol (pre-REP) stage and another combination of cytokines and a pathway inhibitor during the REP stage to control T-cell activation and differentiation while maintaining a 22-day expansion process
- The expansion potential, viability, and phenotypic and functional attributes of the final TIL products were evaluated by a variety of assays at the end of the REP stage
- For single-cell RNA sequencing (scRNA-seq), primary and secondary data analyses were performed using Cell Ranger v7 (10X Genomics) with default parameters. The R package Seurat[6] was used for tertiary analysis and data visualization
- Pseudotime analysis was performed using the R package Monocle3[7] with *IL7R* as the root cells marker gene
- For gene set analysis, the R packages Escape[8] and UCell[9] were used to quantify the gene set scores

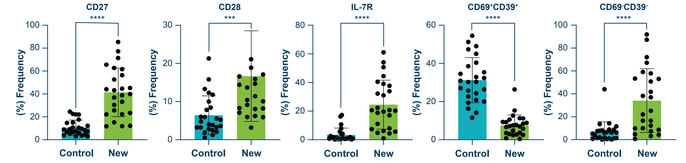
Results

Figure 1. New process increases TIL expansion and viability



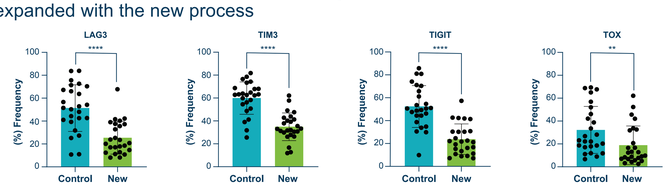
(A) Expansion and viability of TIL at the end of the REP. **(B)** Frequency of CD8+, CD4 (Foxp3-) and CD4 (Foxp3+) T cells in the final product at the end of the expansion process, as measured by flow cytometry.
ns, non-significant; ***p<0.001; ****p<0.0001.

Figure 2. New expansion process increases expression of memory-associated markers and reduces levels of differentiated CD8+ TIL (CD69+CD39+)



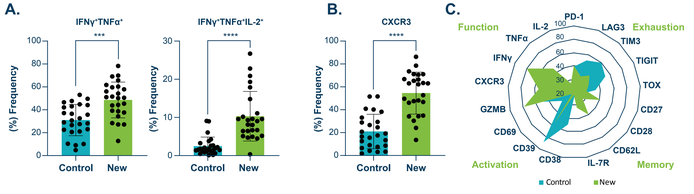
Frequency of memory-associated markers (CD27, CD28, and IL-7R) and CD69+CD39+ and CD69-CD39- expressing CD8+ TIL after control or new expansion process, as measured by flow cytometry.
***p<0.001; ****p<0.0001.

Figure 3. Reduced expression of inhibitory markers and TOX on CD8+ TIL expanded with the new process



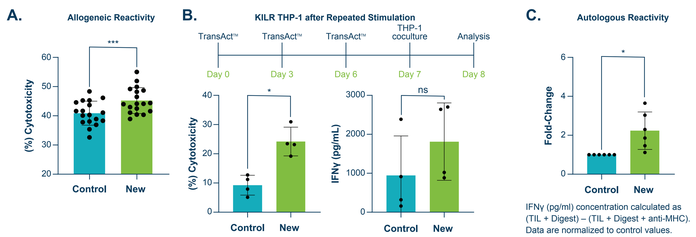
Expression of inhibitory receptors (LAG3, TIM3, TIGIT) and TOX on CD8+ TIL after control or new expansion process, as measured by flow cytometry.
**p<0.01; ****p<0.0001.

Figure 4. Increased polyfunctionality of CD8+ TIL expanded with new process



(A) TIL were stimulated for 5 hours with plate-bound OKT3 followed by staining. Expression of IFNγ/TNFα/IL-2 on CD8+ TIL was measured by flow cytometry. **(B)** CXCR3 expression on unstimulated CD8+ TIL, as measured by flow cytometry. **(C)** Average expression differences for markers associated with T-cell function, exhaustion, memory, and activation on TIL expanded with control or new process, as measured by flow cytometry.
***p<0.001; ****p<0.0001.

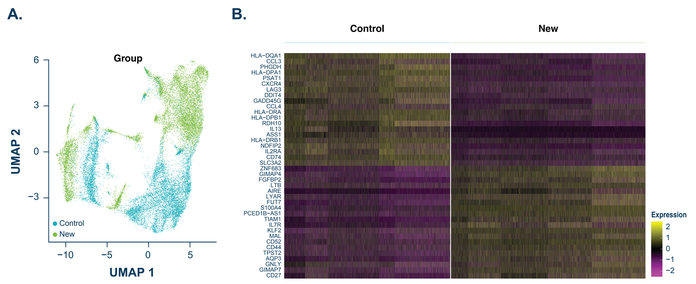
Figure 5. Increased cytotoxicity of TIL expanded with new process even after repeated stimulation



(A) TIL (N=18) were cocultured for 24 hours with KILR® THP-1 cells (Eurofins DiscoverX, Fremont, CA, USA) at a 10:1 effector-to-target cell ratio to measure cytotoxicity in an allogeneic setting. **(B)** TIL were stimulated every 3 days with TransAct™ followed by coculture for 24 hours with KILR® THP-1 cells (N=4). Percent cytotoxicity and expression of IFNγ as measured by ELISA. **(C)** TIL were cocultured with autologous tumor cells for 24 hours followed by IFNγ measurement by ELISA (N=6).
*p<0.05; ***p<0.001.

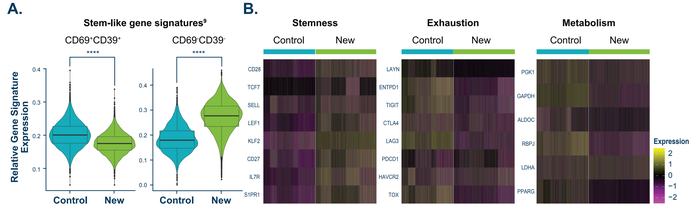
IFNγ (pg/ml) concentration calculated as (TIL + Digest) – (TIL + Digest + anti-MHC). Data are normalized to control values.

Figure 6. New expansion process produces distinct population of TIL



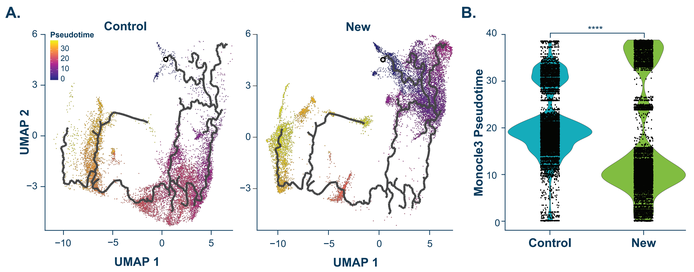
scRNA-seq was performed on TIL expanded using the control or new process (N=5). **(A)** UMAP dimensionality reduction visualization of CD8+ T cell subset colored by group. **(B)** Heatmap visualization of the top 20 differentially expressed (increased and decreased) genes in CD8+ T cell subset.

Figure 7. New expansion process enriches for genes associated with stem-like cells, reduces exhaustion, and enhances metabolic fitness



Gene signature visualizations of TIL expanded with control or new process. **(A)** Violin plots of UCell Score depicting Relative Gene Signature Expression. **(B)** Heatmap visualizations of specific genes of interest.
****p<0.0001.

Figure 8. Pseudotime trajectory analysis shows increased levels of less-differentiated CD8+ TIL with new expansion process



scRNA-seq analysis of differentiation state. **(A)** UMAP dimensionality reduction visualization of CD8+ T cell subset, colored based on Monocle3 defined pseudotime. **(B)** Violin plot of CD8+ T cell subset Monocle3 pseudotime.
****p<0.0001.

Conclusions

- The novel TIL expansion process improves multiple metrics that correlate with both TIL persistence and response, while increasing yield:
  - Enhanced polyfunctionality and cytotoxicity
  - Reduced inhibitory receptor expression
  - Less differentiated and more stem-like phenotype
- These effects may translate into a more vigorous and less differentiated TIL infusion product with improved persistence and cytotoxicity

References

1. Chesney J, et al. Efficacy and safety of lifileucel, a one-time autologous tumor-infiltrating lymphocyte (TIL) cell therapy, in patients with advanced melanoma after progression on immune checkpoint inhibitors and targeted therapies: pooled analysis of consecutive cohorts of the C-144-01 study. *J Immunother Cancer.* 2022 Dec;10(12):e005755.
2. Schoenfeld A, et al. 458 First phase 2 results of autologous tumor-infiltrating lymphocyte (TIL; LN-145) monotherapy in patients with advanced, immune checkpoint inhibitor-treated, non-small cell lung cancer (NSCLC). *J Immunother Cancer.* 2021;9:A486–7.
3. O'Malley D, et al. 492 Phase 2 efficacy and safety of autologous tumor-infiltrating lymphocyte (TIL) cell therapy in combination with pembrolizumab in immune checkpoint inhibitor-naïve patients with advanced cancers. *J Immunother Cancer.* 2021;9:A523–4.
4. Krishna S, et al. Stem-like CD8 T cells mediate response of adoptive cell immunotherapy against human cancer. *Science.* 2020 Dec 11;370(6522):1328-1334.
5. Rosenberg SA, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin Cancer Res.* 2011;17:4550–7.
6. Hao Y, et al. Integrated analysis of multimodal single-cell data. *Cell.* 2021 Jun 24;184(13):3573-3587.e29.
7. Cao J, et al. The single-cell transcriptional landscape of mammalian organogenesis. *Nature.* 2019 Feb;566(7745):496-502.
8. Borcherding N, Andrews J (2022). Escape: Easy single cell analysis platform for enrichment. R package version 1.8.0.
9. Andreatta M, Carmona SJ. UCell: Robust and scalable single-cell gene signature scoring. *Comput Struct Biotechnol J.* 2021 Jun 30;19:3796-3798.

Acknowledgments

- The authors thank the patients who provided tumor tissue for this study
- This study was sponsored by Iovance Biotherapeutics, Inc. (San Carlos, CA)
- Editorial support was provided by Second City Science and funded by Iovance

Disclosures

All authors are employees of, and have stock and/or stock options in, Iovance Biotherapeutics, Inc. (San Carlos, CA)